Fine Mapping of the Locus for Nevoid Basal Cell Carcinoma Syndrome on Chromosome 9q

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The nevoid basal cell carcinoma syndrome is an autosomal dominant disorder characterized primarily by multiple basal cell carcinomas, odontogenic keratocysts, and pits of the palms and soles. Tumor deletion studies and linkage analysis in Caucasians have revealed that the gene is on chromosome 9q. To further refine the location of the nevoid basal cell carcinoma syndrome locus, we tested linkage to this region in three families. Evaluation of recombinants suggested that the nevoid basal cell carcinoma syndrome locus lies in the interval defined distally by D9S127. Our data, together with existing published data defining D9S12 as a proximal flanking marker, refine the location of nevoid basal cell carcinoma syndrome to an 8.3-cM interval. Two of the families studied were African-American and show a notable variation in phenotypic expression in which affected individuals developed few skin cancers. However, despite clinical heterogeneity, our data are consistent with the hypothesis that the same locus is involved in these African-American families. *Key words: Gorlin Syndrome/basal cell nevus syndrome/NBCCS/gene mapping/linkage analysis. J Invest Dermatol 103:178–181, 1994*

The nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterized by multiple basal cell carcinomas, odontogenic keratocysts, pits of the palms and soles, and a variety of other skeletal and developmental abnormalities. Linkage studies in several Caucasian families localized the NBCCS gene to chromosome 9q31 [1–4], and the demonstration of allelic loss at this location in basal cell carcinomas from individuals with NBCCS indicated that the gene probably functions as a tumor suppressor [1]. We have been studying NBCCS in two African-American families in which affected individuals developed few skin cancers, but fully expressed the other components of the disorder [5]. Once the gene is identified and characterized, these families may provide a resource for exploring whether the NBCCS gene in blacks is allelic to that in whites. Fine mapping of the 9q31 region will aid in defining the location of the NBCCS gene. To this end, we have performed genetic linkage analysis in these two families and a third family of Ashkenazi Jewish ancestry. In this study we have confirmed and refined linkage of NBCCS using these families by developing new polymorphisms for D9S29 and utilizing additional polymerase chain reaction–based DNA markers in the region.

MATERIALS AND METHODS

Families Clinical findings in the two African-American families are described in detail elsewhere [5] and summarized here. The probands in these families (2587 and 3060) had jaw cysts, palmar and plantar pits, rare basal cell carcinomas, and significant NBCCS-associated skeletal anomalies. The affected relatives in these pedigrees had jaw cysts and palmar pits. Half of the affected relatives had at least one basal cell carcinoma. Many also had scoliosis, rib anomalies, or other skeletal findings. Pedigrees of these families and of the family of Ashkenazi-Jewish ancestry (N002), are shown in Fig 1.

Family N002: The proband (II-1) was diagnosed at the age of 2½ years with medulloblastoma and treated with x-ray therapy, and subsequently developed basal cell carcinomas and cysts of the jaw at age 14. Invasion of basal cell tumors into the right ear canal, salivary gland, right petrous temporal bone, occipital bone, and dura eventually led to his death at age 40. Family history revealed that I-1 had multiple basal cell carcinomas and jaw cysts. We examined the six offspring of II-1. The oldest (III-1) had two jaw cysts at age 13, palmar and plantar pits, and typical facial features of NBCCS. His sister (III-2), age 12, developed her first jaw cyst at age 10 and basal cell carcinoma at age 11, and had palmar and plantar pits and calcification of the falx cerebri on examination. Individual III-3, age 10, had no history of jaw cysts or basal cell carcinomas and showed none of the signs of NBCCS on dermatologic, dental, radiographic, or ultrasound examinations. The fourth child (III-4) had a jaw cyst at age 8 and a basal cell carcinoma at age 9. He also had palmar and plantar pits. Individual III-5, age 7, had no history of NBCCS-associated findings, nor did our examination reveal anything suspicious for the diagnosis. The youngest child (III-6) was seen at age 6. She had palmar and plantar pits, a rib anomaly, and several probable periorbital basal cell carcinomas.

DNA Marker Analysis DNA was purified from blood lymphocytes or immortalized lymphocyte cell lines by standard procedures. Primer pairs for amplifying microsatellite length variants were as follows: GSN.PCR1.1/GSN.PCR1.2 for GSN(125-147bp) [6]; 9CMP2F/9CMP2R for D9S12 (149-159bp) [7]; A28F/A28R for D9S109 (219-229bp) [8]; D9S12.PCR1.1/D9S12.PCR1.2 for D9S12 (176-194bp) [9]; MX57/MX58 for D9S15 (197-207bp) [10]; Mfd178CA/Mfd178GT for D9S10 (187-203 bp), Mfd135CA/Mfd135GT for D9S53 (116–150 bp) and Mfd189CA/Mfd189GT for D9S106 (99–111 bp) [11]; C3B2-1/C3B2-2 for D9S58 (113–137 bp), D9S119.PCR1.1/ D9S119.PCR1.2 for D9S119 (130–138 bp) [12]; and 2239-1/2239-2 for D9S59 (94–116 bp) [13]. The

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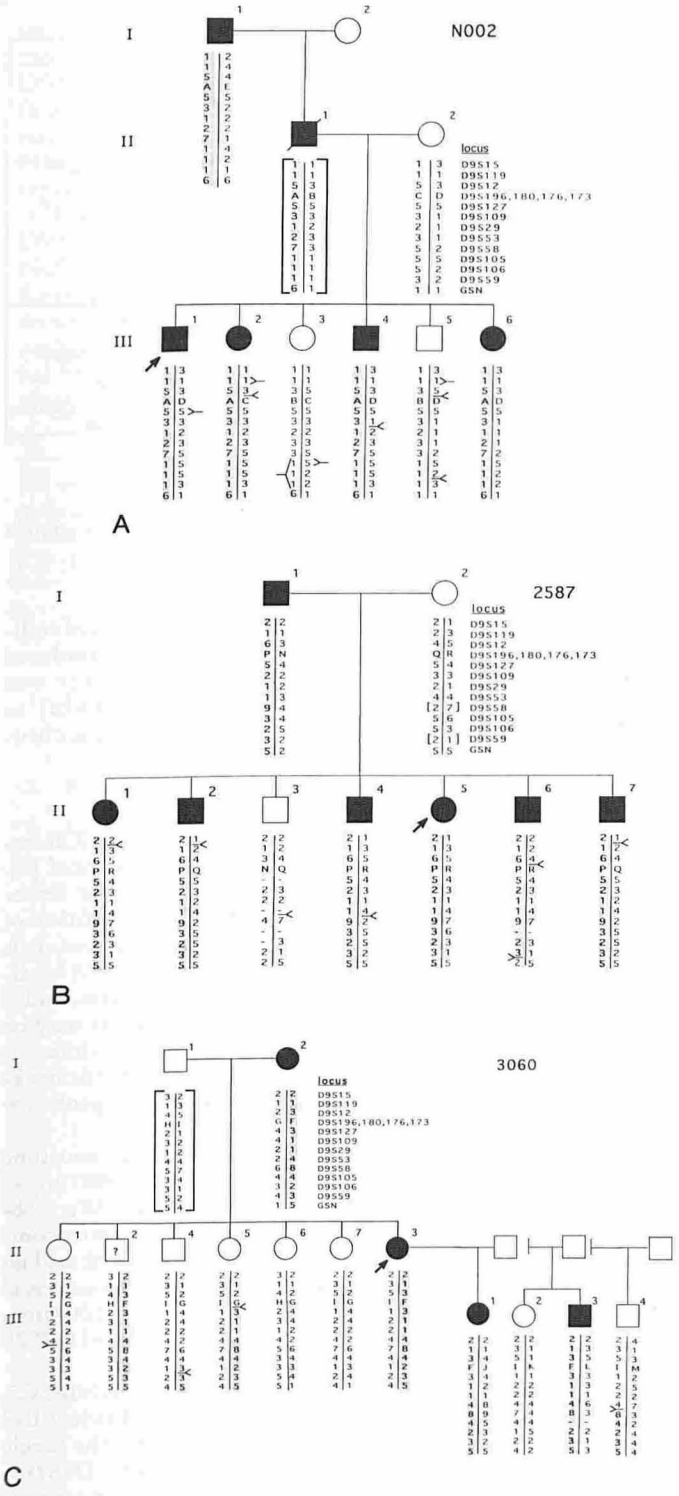

Figure 1. Pedigrees of three families with chromosome 9 haplotypes for members of each family. Families 2587 and 3060 are African-American. Family N002 is Ashkenazi Jewish. Haplotypes were developed based on the order of markers shown. Because no recombination was observed between D9S196, D9S180, D9S176, and D9S173, a haplotype was created. This is indicated by a letter that stands for a unique allele combination (fourth locus from top). ≺, recombination between adjacent loci. ≻, those loci in which phase could not be assigned definitively and hence the exact point of recombination could not be inferred. Shaded chromosome carries the NBCCS disease gene in each family. Inferred haplotypes and genotypes are in *brackets*. *Arrow,* proband for each family. No inferred information was used in the linkage analysis. ?, individual with ambiguous phenotype. This individual was not used for linkage analysis with NBCCS. See text for details.

anonymous microsatellite markers D9S196, D9S180, D9S176 and D9S173 were typed using the primers given in the Genethon Microsatellite Map Catalogue [14]. Specific primer sequences for all markers can be obtained from the Genome Data Base, Johns Hopkins University, Baltimore, MD.

Genomic DNA (200–500 ng) was amplified in a 30-μl reaction with 200 nM primers, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dATP, dGTP, and TTP, 25 mM dCTP, 1.5 μCi ^{32}P-dCTP (3000Ci/mmol), and 0.75 U Taq polymerase (Perkin Elmer Cetus). The thermal cycling protocol for all markers consisted of an initial 95°C, 2 min denaturation, followed by 35 cycles of denaturation at 93°C for 1 min, annealing at 55°C for 1 min, and elongation at 72°C for 1.5 min, and a final 10 min at 72°C. For electrophoretic analysis, reaction volumes were diluted 1 : 1 with formamide, and the amplified DNA was separated on 6% sequencing gels (6M urea) under standard electrophoretic conditions.

Linkage Analysis The inheritance of NBCCS was modeled as a rare, autosomal dominant disorder with full penetrance. Linkage analysis was performed using the computer program LINKAGE Version 5.1 on a VAX workstation. Two-point (disease versus marker) lod scores were calculated using the MLINK subroutine for each marker versus the NBCCS locus. In the absence of published allele frequency data for several of the markers used, alleles were assumed to be of equal frequency. Two five-point linkage analyses (disease versus a fixed map of markers) were performed using the LINKMAP subroutine for the following map: D9S12 — 5.2 cM — D9S176 — 3.1 cM — D9S127 — 0.1 cM — D9S29 — 0.1 cM — D9S53. The order of the markers is taken from published consensus maps for D9S12, D9S127, and D9S29 [15]. Our estimate, using linkage analysis in our families, of the distances between these markers were somewhat different than those in Povey *et al* [15]. However, the distances in our families were more consistent with those proposed by the recent International Workshop on Chromosome 9 for D9S12, D9S176, D9S127, and D9S53 [16]. No information on distance between D9S29 and other markers was provided in that study. The orientation of D9S53 and D9S29 is unresolved. In this study, we have placed D9S53 distal to D9S29 as suggested in Goudie *et al* [17].

RESULTS

Because the initial report of linkage in NBCCS showed no recombination with D9S29 [1], it was important to obtain information with this marker in these families. However, neither of the African-American families were informative for the previously reported TaqI RFLP. Screening with several restriction enzymes revealed polymorphism with EcoRV (McBride OW, in preparation), and both families 3060 and 2587 were informative with this enzyme/probe combination. Interestingly, there was complete linkage disequilibrium between the TaqI and EcoRV polymorphisms in family N002.

Using the EcoRV polymorphism in the two African-American families and the TaqI polymorphism in the Ashkenazi Jewish family, linkage of the gene for NBCCS to D9S29 was confirmed ($Z = 3.3$; $\Theta = 0$). Family-specific lod scores for 14 chromosome 9 markers are shown in Table I. In addition to D9S29, there was no evidence of recombination in any of the families from two-point analysis with D9S12 ($Z = 3.9$), D9S196 ($Z = 4.85$), D9S180 ($Z = 3.9$), D9S176 ($Z = 5.29$), and D9S173 ($Z = 2.62$). Results for D9S15 and D9S119 have not been included in the table, as they were virtually uninformative, except in creating the haplotypes.

To identify the most probable location for the NBCCS locus within the map of linked markers, multipoint analysis was performed for D9S12, D9S176, D9S127, D9S29, and D9S53 and the disease. Due to computational limitations imposed by the large number of alleles for some markers, results were derived by overlapping two four-point analyses using the first four, and then the last four, markers. The order of the markers, and distances between them, was based on previous reports and information from these families (see *Methods*). The maximum lod score ($Z = 6.0$) obtained from this analysis occurred coincident with D9S176. The next highest lod score occurring distal (away from the centromere) to D9S176 was 4.47 (8 cM distal to D9S53, and outside of the map of markers used in the multipoint analysis) (Fig 2).

We also performed haplotype analysis to further examine segregation of the NBCCS gene in individuals where recombination with the disease had occurred. The most probable chromosome 9 haplotypes for each individual are shown in Fig 1, using the marker

Table I. Lod Scores for Each Family with 9q Markers

Family	0.001	0.05	0.10	0.15	$\hat{Z}$	$\hat{\Theta}$
D9S12						
2587	1.81	1.65	1.48	1.31		
3060	2.10	1.93	1.74	1.54		
N002	−0.01	−0.01				
TOTAL	3.90	3.57	3.22	2.85	3.90	0
D9S196						
2587	1.80	1.65	1.49	1.31		
3060	1.50	1.37	1.23	1.08		
N002	1.53	1.40	1.26	1.11		
TOTAL	4.84	4.43	3.98	3.51	4.85	0
D9S180						
2587		(Uninformative)				
3060	2.70	2.49	2.25	2.00		
N002	1.20	1.12	1.02	0.92		
TOTAL	3.91	3.60	3.27	2.93	3.91	0
D9S176						
2587	1.50	1.37	1.23	1.08		
3060	2.58	2.37	2.14	1.90		
N002	1.20	1.07	0.93	0.78		
TOTAL	5.28	4.81	4.30	3.77	5.29	0
D9S173						
2587		(Uninformative)				
3060	2.67	2.45	2.22	1.97		
N002	−0.05	−0.05	−0.03	−0.02		
TOTAL	2.62	2.41	2.19	1.96	2.62	0
D9S127						
2587	0.30	0.26	0.21	0.17		
3060	−.29	1.21	1.30	1.25		
N002	−0.03	−0.01				
TOTAL	−.02	1.45	1.50	1.42	1.50	0.09
D9S109						
2587		(Uninformative)				
3060	−0.89	0.65	0.79	0.79		
N002	−0.07	−0.05	−0.03	−0.02		
TOTAL	−0.96	0.60	0.76	0.77	0.78	0.14
D9S29						
2587	1.20	1.09	0.98	0.85		
3060	1.80	1.63	1.44	1.24		
N002	0.30	0.26	0.21	0.17		
TOTAL	3.30	2.98	2.63	2.26	3.31	0
D9S53						
2587	1.50	1.37	1.23	1.08		
3060	−1.58	0.02	0.20	0.26		
N002	1.80	1.67	1.53	1.38		
TOTAL	1.72	3.06	2.96	2.72	3.06	0.06
D9S58						
2587	1.80	1.65	1.48	1.31		
3060	−3.29	−0.07	0.34	0.50		
N002	1.80	1.67	1.53	1.38		
TOTAL	0.31	3.25	3.36	3.19	3.40	0.09
D9S105						
2587	1.20	1.09	0.98	0.85		
3060		(Uninformative)				
N002	−0.03	−0.03	−0.03	−0.01		
TOTAL	1.17	1.06	0.95	0.84	1.17	0
D9S106						
2587	1.50	1.37	1.23	1.08		
3060	−3.29	−0.07	0.34	0.50		
N002	−0.03	−0.03	−0.02	−0.01		
TOTAL	−1.82	1.27	1.55	1.57	1.58	0.13
D9S59						
2587	1.80	1.65	1.48	1.31		
3060	−6.29	−1.35	−0.61	−0.24		
N002	−0.03	−0.02	−0.02	−0.01		
TOTAL	−4.52	0.28	0.85	1.06	1.10	0.18
GSN						
2587	−0.02	0.46	0.57	0.58		
3060	−3.60	−0.35	0.09	0.28		
N002	−1.19	0.39	0.58	0.63		
TOTAL	−4.81	0.51	1.24	1.49	1.53	0.18

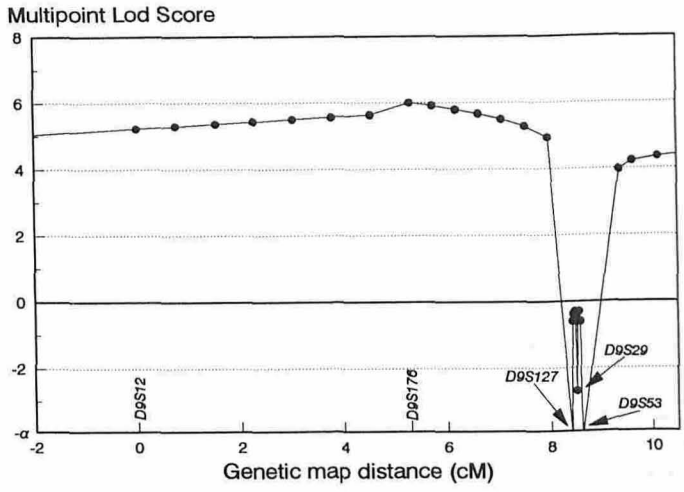

Figure 2. Multipoint linkage analysis in three families of NBCCS versus a map of five polymorphic DNA markers on chromosome 9q.

order discussed above and indicated in the figure. Diagnosis of individual II-2 in family 3060 was uncertain [5], and he was considered unknown for linkage analysis. Because his affected mother was homozygous for markers proximal (toward the centromere) to D9S12, we cannot determine if he inherited an intact disease chromosome.

DISCUSSION

There are clinical differences in the presentation of NBCCS between Blacks and Caucasians, as described by Goldstein *et al* [5]. Specifically, African-Americans with this disease have far fewer skin cancers than affected Caucasians, with possible exacerbation of the extra-cutaneous manifestations of the disease. However, it is clear from the data presented here that the gene for NBCCS localizes to the same region on 9q in both Blacks and Caucasians, and is likely to be due to the same locus. The clinical differences may be accounted for by different alleles at the same locus (i.e., different mutations in the NBCCS gene), or differences in the genetic background on which the NBCCS gene is expressed (i.e., genes for pigmentation).

Our data are consistent with the order of markers on chromosome 9q being cen—D9S12—[D9S176,D9S180,D9S173,D9S196]—[D9S127,D9S109]—[D9S29,D9S53]—D9S58—tel. We observed no recombination between D9S127 and D9S109; no recombination between D9S176, D9S180, D9S173, and D9S196; and no recombination between D9S29 and D9S53. However, Goudie *et al* [17] state that the order cen—D9S29—D9S53—tel is 100 times more likely than the inverse order, and we have thus placed D9S29 proximal to D9S53 for the multipoint linkage analysis.

Our data establish D9S127 as a distal flanking marker to NBCCS, based substantially on the haplotype for unaffected individual II-5 of family 3060 (Fig 1C). This individual accounts for the single recombination between the NBCCS gene and D9S127, D9S109, and D9S53 identified by two-point linkage analysis. The parental genotypes for D9S29 were uninformative, both parents being 2-1 at this locus; therefore two-point analysis was unable to identify a recombination. However, the disease haplotype was present in II-5 for all markers distal to the cosegregating group of D9S196, D9S180, D9S176, D9S173, and therefore recombination with D9S29 in this individual is probable. As this individual had none of the findings associated with NBCCS upon examination, the implication is that the NBCCS locus must lie proximal to D9S127.

A proximal flanking marker to the NBCCS gene cannot be derived from the two-point linkage analysis in these families because D9S12 showed complete linkage with NBCCS, and we have therefore constructed conservative haplotypes (where recombination occurs on the non-disease chromosome) consistent with this analy-

sis. However, alternative haplotypes of individuals III-2 and III-5 in family N002 can be derived that invoke recombination distal to D9S12 on the paternally derived disease chromosome. For example, in affected person III-2, the alternative haplotype with the recombination occurring between D9S12 and distal markers places the NBCCS locus distal to D9S12. Such an event has been previously reported in other families [2,3].

These data, taken together, place the NBCCS locus proximal to D9S127. Evidence from other studies [3,18] and haplotyping in N002 in this paper support assignment of D9S12 as a proximal flanking marker. Our data, therefore, define an 8.3-cM interval around the NBCCS locus. The markers D9S127 and D9S12, together with D9S176, D9S196, and D9S180, will be extremely useful in prenatal diagnosis in informative families, and for further physical mapping strategies aimed at isolating the NBCCS gene.

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